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Data Evaluation Report on the Chronic Toxicity of DPX-MAT28 Technical (Aminocyclopyrachlor) to Freshwater Invertebrates - Daphnia sp.

PMRA Submission Number {.....} EPA MRID No. 47560129

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	358148
	OECD Data Point	{.....}
	EPA MRID	47560129
	EPA Guideline	OPPTS 850.1300

Test material: DPX-MAT28 Technical **Purity:** 92.2%
Common name Aminocyclopyrachlor
Chemical name:
IUPAC: 6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
CAS: 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
CAS No.: 858956-08-8
Synonyms: None reported

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature: Christie E. Padova
Date: 07/20/09

Primary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.
Signature: Teri S Myers
Date: 07/24/09

Secondary Reviewer: Anita Ullagaddi
EPA/OPP/EFED/ERB1
Signature: Anita
Date: 10/08/09

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
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Use Site Category:	{.....}	[For PMRA]
EPA PC Code	None assigned	

Date Evaluation Completed: 10/08/09

CITATION: Gallagher, S.P., T.Z. Kendall, and H.O Krueger. 2008. DPX-MAT28 Technical: A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*). Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Study No. 112A-238B. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, DE. Study initiated February 13, 2008 and submitted August 29, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the chronic toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

2084705

The 21-day-chronic toxicity of DPX-MAT28 Technical (aminocyclopyrachlor) to *Daphnia magna* was studied under static renewal conditions. Daphnids were exposed to DPX-MAT28 Technical at nominal concentrations of 0 (negative control), 0.38, 0.75, 1.5, 3.0, and 6.0 mg ai/L. Reviewer-calculated time-weighted average (TWA) concentrations were identical to mean-measured concentrations and were <0.00215 (<LOD, control), 0.37, 0.73, 1.5, 3.0, and 6.0 mg ai/L, respectively. The 21-day LC_{50} for adult mortality was >6.0 mg ai/L. However, non dose-response mortalities were observed in the 0.37, 0.73, and 1.5 mg a.i./L of 40%, 30, and 40%, respectively. Therefore, a NOAEC could not be established.

This study is scientifically sound but is classified as supplemental. Due to the high mortality observed in the first three treatment levels, the resulting endpoints are not a reliable estimate of the chronic toxicity to daphnids. Additionally, immobility was observed in all treatment levels of the second range-finding test; however, data for the range-finding tests were not provided. Finally, the Agency has evidence of sublethal effects and mortality occurring in a daphnid acute toxicity test providing a NOAEC of 3.7 mg a.i./L; this comparison suggests a possible issue with the health of the test organisms in this chronic study.

Results Synopsis

Test Organism Age (eg. 1st instar): First instar, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static renewal

LC_{50}: >6.0 mg ai/L 95% C.I.: N/A
NOAEC: N/A
LOAEC: 0.37 mg ai/L

Endpoints affected: mortality

I. MATERIALS AND METHODS

GUIDELINE(S) FOLLOWED: U.S. EPA OPPTS No. 850.1300 (1996)
OECD Guideline No. 211 (1998)

The following deviation from U.S. EPA OPPTS Guideline 850.1300 was observed:

Data were not provided for the range-finding test in which immobility was observed in all treatment levels. The guideline states that the study sponsor must submit all data developed by the test that are suggestive or predictive of chronic toxicity and all associated toxicological manifestations.

This deviation affects the acceptability classification of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the GLP Standards as published in the 40 CFR, Part 160 with the following exception: periodic analysis of well water for potential contaminants.

A. MATERIALS:

1. Test Material DPX-MAT28 Technical (aminocyclopyrachlor)

Description: Solid

Lot No./Batch No.: 009

Purity: 92.2%

Stability of compound under test conditions: Verified: concentrations of DPX-MAT28 Technical (aminocyclopyrachlor) were maintained within 5% at each level.

Physicochemical properties of Aminocyclopyrachlor.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

2. Test Organism:

Species:	*Daphnia magna*, first instar, <24 hours old *EPA and OECD recommend Daphnia magna*
Age of the parental stock:	Cultures were held 26 days prior to neonate collection *EPA recommends that young daphnids ≤24 hours old from a separate parental culture be used*
Source:	Laboratory culture *EPA requires all test organisms must be produced from laboratory reared culture that has been maintained for at least 21 days at test conditions in dilution water with renewal of the culture medium at least three times per week.*

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The concentrations selected for use in the definitive study were based upon results of exploratory range finding data and in consultation with the Sponsor. The first trial study was terminated after 12 days as >20% control mortality occurred (animals appeared to be diseased or stressed). The second trial study was conducted for 21 days; however, immobility in all treatment levels prevented determination of a NOAEC and LOAEC.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period:	26 days	Each parental daphnid had produced at least one previous brood, and had produced an average of ≥3 young per adult per day over the 7-day period preceding the test. The progeny form four adults were used in the test.
Conditions: (same as test or not)	Generally same as test, although it was not reported if daphnia were maintained under flow-through or static renewal conditions.	
Feeding:	A mixture of yeast, cereal grass media, and trout chow (YCT), as well as a suspension of the freshwater green alga, (*Pseudokirchneriella subcapitata*).	*EPA recommends that prior to testing, daphnids that are at least 10-12 days old (those that have had at least one brood) should be separated from the culture, put in separate container and maintained for at least 21 days to insure that good health conditions are present*
Health (any mortality observed):	The adults exhibited no signs of disease or stress during the holding period. In addition, no ephippia were produced during the holding period.	
Test condition: static renewal/flow-through:	Static renewal	*(EPA requires consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Type of dilution system- for flow through method.	N/A	
Renewal rate for static renewal	Three times weekly (Monday, Wednesday, and Friday) during the first 2 weeks of the test, and twice during the last week of the test.	
Aeration, if any	No supplemental aeration was reported.	*EPA recommends test chambers should not be aerated*

Parameter	Details	Remarks *Criteria*
Duration of the test	21 days	*Recommended duration is 21 days.*
Test vessel Material: (glass/stainless steel) Size (for growth and reproduction/survival test): Fill volume:	Glass beakers 250 mL 220 mL (*ca.* 7.8-cm depth)	Each chamber was loosely covered with a plastic petri dish. *1. Recommended Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Recommended Size: 250 ml with 200 ml fill volume; 100 ml with 80 ml fill volume* *OECD guideline recommends that parent animals be maintained individually; one per vessel, with 50 - 100 ml of medium in each vessel.*
Source of dilution water	Moderately-hard freshwater was obtained from a 40-m deep well located at the laboratory. The well water was sand-filtered, aerated, filtered again (0.45-µm), and UV-sterilized prior to use. The following parameters were measured weekly for 4 weeks preceding the test: specific conductance 300 to 310 µmhos/cm, hardness 132 to 148 mg/L as $CaCO_3$, alkalinity 180 to 186 mg/L as $CaCO_3$, and pH 8.1 to 8.3.	*Recommended source of dilution water includes unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature	 132 to 148 mg/L as $CaCO_3$ 8.1 to 8.4 ≥7.0 mg/L (≥77% saturation) 19.0 to 20.8°C	It was noted that DO levels were decreasing during the first week, so the test chambers were periodically cleaned and additional DO measurements were performed beginning on day 10.
Conductivity Alkalinity Total Organic Carbon Particulate matter Inorganic analytes Pesticides Chlorine interval of water quality measurements:	315 to 361 µmhos/cm 176 to 182 mg/L as $CaCO_3$ <2 mg C/L Not reported Not reported Not reported Not reported Temperature was measured in two alternating chambers for each level at test initiation, in old and new solutions at each renewal period, and at test termination. Temperature was also monitored continuously in a container of water adjacent to the chambers. DO and pH were measured in freshly-prepared batch solutions for each level at test initiation and each renewal period, and in old media from two alternating chambers of each level. Hardness, alkalinity, and conductivity were measured in batch solutions of the negative control and highest test level at test initiation, and on Days 8 and 15, and from pooled replicate solutions at test termination. TOC was measured in the dilution water at test initiation, on Days 8 and 15, and from pooled replicate solutions at test termination.	***Recommended hardness:*** *160 to 180 mg/L as $CaCO_3$; OECD recommends > 140 mg/L as $CaCO_3$* ***Recommended pH****: 7.6 to 8.0 pH should not deviate by more than 1.0 unit for more than 48 hours. OECD recommends that pH range be 6 - 9 and does not vary more than 1.5 units in any one test.* ***Recommended dissolved oxygen:*** *renewal should not drop below 50% for more than 48 hours. Recommended flow-through: ≥60% throughout test.* ***Recommended temperature****: 20°C ± 2°C.; should not deviate from 20°C by more than 5°C for more than 48 hours. OECD recommends a range of 18 - 22°C; temperature should not vary more than ± 2°C* *OECD guideline recommends that total organic carbon < 2 mg/L*

Parameter	Details	Remarks *Criteria*
Number of replicates: For growth and reproduction: For survival test:	20 replicate chambers for the control level and 10 replicate chambers for each treatment level Same	There were five toxicant levels spaced by a separation factor of 2. *Number of replicates should include a control(s) and at least 5 test concentrations; dilution factor should not be greater than 50%.* *OECD recommends that at least 5 test concentrations be used in a geometric series with a separation factor not exceeding 3.2.*
Number of organisms: For growth and reproduction: For survival test:	1 daphnia per replicate Same	Fulfills OPPTS guidelines for a static renewal system. *Recommended number of organisms include 22 daphnids/test concentration;* *7 test chambers should contain 1 daphnid each, and 3 test chambers contain 5 daphnids each.* *OECD recommends holding a minimum of 10 daphnids individually for static tests.* *For flow-through tests, 40 animals should be divided into 4 groups of 10 animals at each test concentration.*
Treatment Concentrations: nominal: mean-measured and TWA (reviewer-calculated):	0 (negative control), 0.38, 0.75, 1.5, 3.0, and 6.0 mg ai/L <0.00215 (<LOD, control), 0.37, 0.73, 1.5, 3.0, and 6.0 mg ai/L	Concentrations of DPX-MAT28 Technical were verified at 0, 3 (old and new), 6 (old), 10 (new), 13 (old), 17 (new), and 21 (old) days. Samples were direct-injected into an HPLC with UV (220 nm) detection. TWA concentrations were reviewer-calculated and were identical to mean-measured concentrations (see copy of Excel worksheet in Appendix II).

Parameter	Details	Remarks *Criteria*
(type, percentage, if used)	N/A	*Solvent concentration should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD recommends #0.1 ml/L of solvent.*
Lighting	16 hours light/8 hours dark, with 30-minute low-light transition periods	Light intensity at test initiation was 235 lux over the surface of the test water at study initiation. *Recommended photoperiod is 16 hours light and 8 hours of dark.*
Recovery of chemical: Frequency of measurement: LOD: LOQ:	94.6 to 102% of nominal Days 0 (new), 3 (old and new), 6 (old), 10 (new), 13 (old), 17 (new), and 21 (old) 0.00215 mg ai/L 0.200 mg ai/L	Based upon sample recoveries.
Positive control {if used, indicate the chemical and concentrations}	None tested	
Other parameters, if any Feeding:	During the test, daphnids were fed once daily a mixture of yeast, cereal grass media, and trout chow (YCT), as well as a suspension of the freshwater green alga, (*Pseudokirchneriella subcapitata*).	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Data endpoints measured (list)	- Parental survival - Clinical signs of toxicity - Presence of eggs in the brood pouch - Aborted eggs, males, or ephippia - Neonate production - Terminal length and dry weight of surviving first-generation daphnia	*Recommended endpoints measured:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.*
Observation intervals	Parents were observed daily during testing. With the onset of reproduction, neonates were counted and discarded every Monday, Wednesday, and Friday. Growth measurements were determined on Day 21.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY AND CLINICAL SIGNS OF TOXICITY:

After 21 days, survival averaged 90, 60, 70, 60, 90, and 90% in the negative control, 0.37, 0.73, 1.5, 3.0, and 6.0 mg ai/L levels, respectively (mean-measured concentrations). No statistically-significant differences from the control were observed. The NOAEC for parental survival was 6.0 mg ai/L, and the 21-day EC_{50} for immobility was >6.0 mg ai/L.

No treatment-related signs of toxicity were indicated at any treatment level. The NOAEC for clinical signs of toxicity was 6.0 mg ai/L.

Table 3: Effect of DPX-MAT28 Technical (Aminocyclopyrachlor) on Growth and Survival of Daphnia sp.

Treatment Concn. Mean-measured (and nominal), mg ai/L[(a)]	Cumulative Mortality (dead or immobile)		Mean Day of First Brood	Mean No. Young Per Surviving Adult	Mean Total Body Length, mm ± SD	Mean Dry Weight, mg ± SD
	No.	%				
Negative control	2	10	7	296	5.3 ± 0.23	1.55 ± 0.33
0.37 (0.38)	4	40	7	285	5.1 ± 0.21	1.53 ± 0.20
0.73 (0.75)	3	30	7	306	5.4 ± 0.26	1.71 ± 0.16
1.5 (1.5)	4	40	7	305	5.4 ± 0.17	1.72 ± 0.22
3.0 (3.0)	1	10	7	292	5.3 ± 0.19	1.65 ± 0.19
6.0 (6.0)	1	10	7	310	5.4 ± 0.12	1.64 ± 0.39
NOAEC, mg ai/L	6.0		6.0	6.0	6.0	6.0
LOAEC, mg ai/L	>6.0		>6.0	>6.0	>6.0	>6.0
LC/EC_{50} (with 95% C.I.), mg ai/L	>6.0		>6.0	>6.0	>6.0	>6.0

[(a)] Reviewer-calculated TWA concentrations were identical to mean-measured concentrations (refer to Appendix II).

B. EFFECTS ON REPRODUCTION AND GROWTH:

There were no treatment-related effects upon any reproductive parameter. In addition, no aborted eggs and no males or ephippia were produced at any level during the study. For all levels, the first day of brood release was Day 7, and the mean number of young produced per surviving adult ranged from 285 to 310. Fourteen immobile daphnids were counted for a single replicate vessel (Replicate I) on Day 17; however, this was an isolated event and not considered to be treatment-related. The NOAEC for all reproductive endpoints was 6.0 mg ai/L.

There were no treatment-related effects upon the growth of surviving adults. For all levels, mean body lengths ranged from 5.1 to 5.4 mm, and mean dry body weights ranged from 1.53 to 1.72 mg. The NOAEC for both growth endpoints was 6.0 mg ai/L.

C. REPORTED STATISTICS:

Statistical comparisons were made upon parent survival, the number of live young produced per surviving adult, and the length and dry weight of surviving daphnids at study termination. Survival data (discrete-variable data) were analyzed using Chi-square and Fisher's Exact tests to identify treatment groups that were statistically significant from the controls ($p \leq 0.05$). Reproduction and growth data (continuous-variable data) were first analyzed for normality using the Shapiro-Wilk or Chi-Square test and for homogeneity of variances using Levene's test ($p=0.01$). All data met these assumptions, and were subsequently compared to the pooled control using ANOVA followed by either Bonferroni's t-test or Dunnett's test ($p \leq 0.05$). The LOAEC, NOAEC, and MATC were reported based upon significance of the data. All statistical tests were performed using mean-measured concentrations and SAS or TOXSTAT statistical software.

The 21-day LC/EC_{50} values were estimated to be greater than the highest treatment level.

LC_{50}: >6.0 mg ai/L 95% C.I.: N/A
NOAEC: 6.0 mg ai/L
LOAEC: >6.0 mg ai/L
MATC: >6.0 mg ai/L

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Although the Fisher's Exact Test revealed no significant differences in mortality between the control and treatment groups, the reviewer visually observed biologically significant mortality events in the three lowest treatment levels (0.37, 0.73, and 1.5 mg a.i./L). Therefore, a NOAEC could not be calculated. The reviewer statistically verified that there were no significant reproductive or growth effects for the only treatment group exhibiting a reduction from control (i.e., 0.37 mg ai/L). A t-test was used to compare the control group to the lowest treatment group for total offspring per surviving adult, length, and weight. Additionally, Fisher's Exact Test was conducted to confirm that there were no significant effects on immobility at any level. Although no significant growth or reproductive effects were observed, the low parental survival reduces the reliability of the chronic toxicity estimates.

LC_{50}: >6.0 mg ai/L 95% C.I.: N/A
NOAEC: N/A
LOAEC: 0.37 mg ai/L

Endpoints affected: mortality

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions did not agree with the study authors'. The parental mortality occurring in the three lowest treatment levels was considered biologically significant, which precluded determination of the NOAEC.

All validity requirements were met. Specifically, 1) ≤20% of the control organisms appeared to be immobilized, stressed, or diseased during the test; 2) each surviving control daphnid produced an average of >60 young; and 3) no ephippia were produced by control animals.

TWA concentrations were reviewer-calculated (refer to associated Excel worksheet in Appendix II) and were identical to mean-measured concentrations reported by the study authors. TWA concentrations were calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C_{TWA} is the time-weighted average concentration,
C_j is the concentration measured at time interval j (j = 0, 1, 2,...n)

t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., t_0 = 0 hours (test initiation), t_1 =24 hours, t_2 =96 hours).

A 50 mg ai/L primary stock solution was prepared in dilution water by sonicating for 5 minutes followed by inverted mixing at least 20 times. The primary stock solution appeared clear and colorless, and from this proportional dilutions were prepared in dilution water. The test solutions were mixed with a stainless steel whisk for *ca.* 2 to 3 minutes. The test solutions appeared clear and colorless at test initiation and were clear with a slight green tint (algal growth) at test termination.

The in-life portion of the definitive study was conducted from April 8 to May 1, 2008.

G. CONCLUSIONS:

This study is scientifically sound and is considered supplemental. Due to the biologically significant mortality in the three lowest treatment levels, a NOAEC could not be determined.

LC_{50}: >6.0 mg ai/L 95% C.I.: N/A
NOAEC: N/A
LOAEC: 0.37 mg ai/L

Endpoints affected: mortality

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1300: *Daphnid Chronic Toxicity Test.*

Organization for Economic Cooperation and Development. 1998. OECD Guidelines for Testing of Chemicals. Guideline 211: *Daphnia magna Reproduction Test.*

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	2	
1	0.37	10	4	
2	0.73	10	3	
3	1.5	10	4	
4	3.0	10	1	
5	6.0	10	1	

Total Offspring Production			Length			Dry weight		
control	0.37	t-test	control	0.37	t-test	control	0.37	t-test
292	.	p-value	5.3	5	p-value	1.81	1.65	p-value
.	267	**0.1625**	5.3	4.8	**0.0920**	0.37	1.39	**0.8895**
259	285		5	5		1.51	1.86	
271	302		5.2	5.2		1.61	1.38	
297	289		5.3	5		1.7	1.55	
278	.		5.7	5.4		1.56	1.35	
318	283		5.2			1.65		
.	.		5.3			1.63		
294	.		5.8			1.61		
302	285		5.3			1.6		
288	**285**		5.1			1.28		
301			5			1.68		
336			5.2			1.67		
311			5.5			1.53		
294			4.9			1.56		
307			5.2			1.5		
299			5			1.66		
295			5.3			1.97		
308								
283								
296								

APPENDIX II: COPY OF REVIEWER'S TWA CALCULATIONS USING EXCEL:

As solutions were not sampled before and after the renewals, a standard TWA formula was used, and the denominator was adjusted to represent the sum of the averaged intervals.

nominal	time,		measured	mean measured	
mg ai/L	days		mg ai/L	mg ai/L	
0.38	0	fresh	0.375	0.375	
	3	old	0.371	0.371	
	3	old	0.371		
	3	fresh	0.384	0.384	
	6	old	0.374	0.3745	
	6	old	0.375		
	10	fresh	0.374	0.374	
	13	old	0.37	0.371	
	13	old	0.372		
	17	fresh	0.381	0.381	
	21	old	0.369	0.369	
	21	old	0.369		
				TWA=	**0.375**
				% VARIATION	**4**
0.75	0	fresh	0.737	0.737	
	3	old	0.736	0.735	
	3	old	0.734		
	3	fresh	0.742	0.742	
	6	old	0.729	0.7245	
	6	old	0.72		
	10	fresh	0.734	0.734	
	13	old	0.723	0.723	
	13	old	0.723		
	17	fresh	0.731	0.731	
	21	old	0.709	0.7095	
	21	old	0.71		
				TWA=	**0.729**
				% VARIATION	**4**
1.5	0	fresh	1.48	1.48	
	3	old	1.47	1.47	
	3	old	1.47		
	3	fresh	1.48	1.48	
	6	old	1.44	1.435	
	6	old	1.43		
	10	fresh	1.49	1.49	
	13	old	1.47	1.47	

	13	old	1.47		
	17	fresh	1.51	1.51	
	21	old	1.46	1.46	
	21	old	1.46		
				TWA=	**1.475**
				% VARIATION	**5**
3	0	fresh	2.96	2.96	
	3	old	3.01	3.01	
	3	old	3.01		
	3	fresh	3.01	3.01	
	6	old	2.92	2.925	
	6	old	2.93		
	10	fresh	2.95	2.95	
	13	old	2.92	2.915	
	13	old	2.91		
	17	fresh	3.03	3.03	
	21	old	2.92	2.925	
	21	old	2.93		
				TWA=	**2.967**
				% VARIATION	**3**
6	0	fresh	5.96	5.96	
	3	old	5.97	5.945	
	3	old	5.92		
	3	fresh	6.06	6.06	
	6	old	5.96	5.94	
	6	old	5.92		
	10	fresh	5.99	5.99	
	13	old	5.91	5.905	
	13	old	5.9		
	17	fresh	6.11	6.11	
	21	old	5.92	5.92	
	21	old	5.92		
				TWA=	**5.982**
				% VARIATION	**3**